IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Appl. No.	:	**10/007,459**
Applicants	:	**David L. Lewis et al.**
Filed	:	**11/07/2001**
Art Unit	:	**1635**
Examiner	:	**Gibbs, Terra C.**
Docket No.	:	**Mirus.030.04**

For: **Inhibition of Gene Expression by Delivery of Small Interfering RNA to Post-Embryonic Animal Cells *In Vivo***

Commissioner of Patents
PO Box 1450
Alexandria, VA 22313-1450

DECLARATION UNDER 37 C.F.R. §1.131

Dear Commissioner:

We, Jon A. Wolff, James Hagstrom, Hans Herweijer, David Lewis, Aaron Loomis, and Vladimir Budker, inventor(s) of the above captioned Application, hereby declare as follows:

1. We are inventors of the captioned application.

   Jon A. Wolff and Vladimir Budker are the inventors of the process for intravascular injection of nucleic acid into a vessel wherein the volume and rate of the injection results in delivering the nucleic acid from inside the vessel to into an parenchymal cell of claim 1.

   Dave Lewis, Jon A. Wolff, Vladimir Budker, Hans Herweijer, James E. Hagstrom, and Aaron Loomis are inventors, separately or together, on claims 11 and 14-18.

   Jon A. Wolff and Vladimir Budker are also authors of the cited reference, Zhang et al. Human Gene Therapy 1999, Vol. 10, p. 1735-1737.

2. Applicants' *in vivo* nucleic acid delivery process of claim 1 was conceived prior to the effective date of the Office Action prior art references, Zimmer (Methods, 1999) and Zhang et al. (Human Gene Therapy 1999).

3. We hereby submit photocopies of laboratory notebook pages from the notebooks of researchers working under our direction, dated January 19-22, 1999, and February 10-12 and 19-24, 1999, describing mixing nucleic acid with a polymer to form a complex having a zeta potential that is less negative than the nucleic acid and injecting the complex into a vessel in a mammal in a volume and at a rate sufficient to delivery the nucleic acid to an extravascular cell, prior to the publication date of the Zimmer (Methods, 1999) and Zhang et al. (Human Gene Therapy 1999) cited in the Office Action.

   Page 1 of the attached photocopies shows a description of a polycationic polymer used to form a complex with the nucleic acid. Because the polymer is cationic (at the nitrogen atoms), the polymer-nucleic acid complex less negative than the zeta potential of the nucleic acid alone. This polymer, MC00016 (or MC16), was used in the other experiments shown in this declaration.

   Pages 2, 5, 8 of the attached photocopies show descriptions of complex formation between nucleic acid and polymer MC16.

   Pages 2, 3, 5, 6, 8 and 9 of the attached photocopies show descriptions of the injection parameters.

   Pages 4, 7, 10 and 11 of the attached photocopies show effective liver delivery following injection into tail vein.

4. It is known to us that the process performed in the notebook pages results in delivery of the nucleic acid to extravascular cells as described in the above captioned specification.

5. Development of the nucleic acid complex delivery process occurred with due diligence from conception to the filing of the application.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

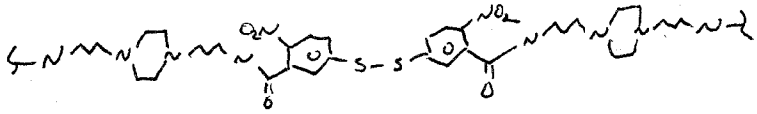
Jon A. Wolff date	(deceased) Vladimir G. Budker date
James E. Hagstrom 8/21/08 date	Dave Lewis 8/21/08 date
/Aaron G. Loomis/ 8/20/2008 Aaron Loomis date	8/21/08 Hans Herweijer date

Mirus Corporation Compound Sheet

MC Number	Lot Number	Date Submitted
MC00016		1/19/98

Chemical Name

Mol. Formula	Structure
Mol. Weight	
Factor	
Compound Class: Cationic Polymer	
Project: Particle Formation	
Submitted by: S. Monahan	
Notebook: 2-048-2	
Amt. Submitted: 1.2 mg ~~3.0 mg~~	
Amt. Remaining: 2.5 mg	
Appearance: red solid	
Approved by	
Release Date	Lit. Ref



Elemental Analysis		Analytical	Distribution
Calculated	Found	1H NMR ✓	
		HPLC	
		Purity	
Solubility		Other 12-14,000 dialysis	

M16 [BATCH M3] VS. DNA + PLL-DNA IN VIVO

MIX UP FORMULATIONS AS FOLLOWS.

1- 200μgs DNA IN 2 mls H_2O

2- 200μgs DNA IN 2mls H_2O

COMPACT WITH ~3x CHARGE M16 (420μgs)

3- 200μg DNA IN 2mls H_2O

COMPACT WITH 3x CHARGE PLL34K - (380μgs)

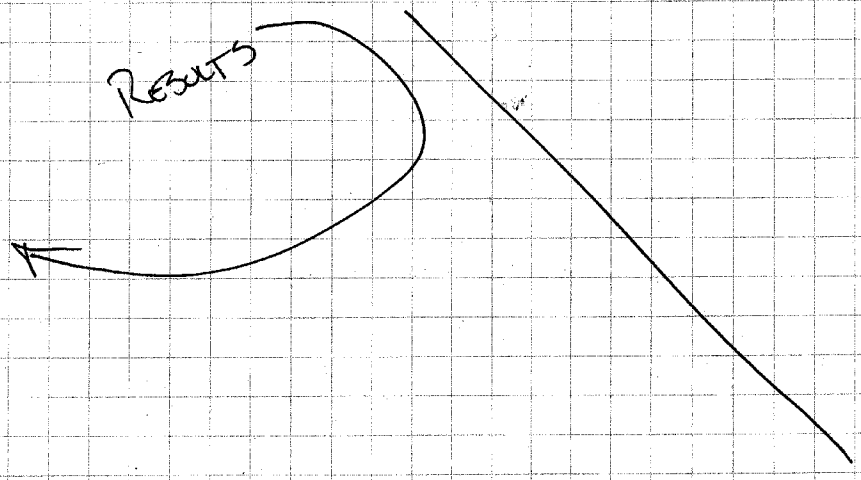
MIX WITH RINGERS SOLUTION TO 2½ mls

HIGH PRESSURE TAIL VEIN INJECT

HARVEST @ 24 HOURS

MICE ARE OLD

HIGH TAIL EXPRESSION IS A GOOD INDICATOR OF A BAD INJECTION



1/21/99

Project: IM IV (InVasc) Gut Other: EXP#: InVascHP #10 Date: 1/19/99

Method: LPTail (HPTail) LPPortal HPPortal LPGut HPGut IM
2.5 Mil's - NP

Charge to: ATP PrdDev Other

Researcher(s): Sean

Particle/Compound Being Tested: Naked DNA vs MIRUS MC16 vs Poly lysine

Delivery Medium: Saline PBS Glu Man H2O Ringers other

Stability Issues? —

Hazardous Material? —

Other Cautions? —

Time Point(s): 24 Hr Harvest

# of Samples: 3 x 3 = 9 total Animals

Explanation/Code:

End Result: Expression: (Luciferase) BetaGAL GFP Ligand/SolRecep Ab Screen other

Distribution: Fluorescence Radiation other

Organs to Assay: LIVER LR / MC TAIL
HEART

Notes:

Procedure Notes:

Start Time:

Individual Animals:

Inj Notes

Animal

#1 - Good
#2 - BAD
#3 - OK
} Naked DNA

#4 OK
#5 Great Fast
#6 Great
#7 Great
} MIRUS MC16

#8 2nd foot then 0.5 ml end
#9 1.5 then 1.0 more moved - Quickly 1.0
} Poly lysine

Procedure Performed by:

Date:

1/20/99 INVASC HP T10

B 9507 SER. # 1000

MEASUREMENT ROUTINE

20 JAN 99 18:00 V.2.03

PROTOCOL NO. : 5	NAME : 50/50-10S
VOLUME INJ. 1 [ul]	50
VOLUME INJ. 2 [ul]	50
SEQUENCE OF INJECTIONS	1->2
DELAY TIME INJ 1/INJ 2 [s]	2.0
MEASURE BACKGROUND	YES
MEASURING TIME BKG [s]	0.5
AUTOMATIC BKG SUBTRACT	NO
MAX. BACKGROUND [RLU/s]	50
DELAY LAST INJ./MEAS. [s]	0.5
MEASURING TIME [s]	10.0

No diluted 10 fold - direct from open sample

COMMENT : TAIL

Group	SAMPLE	RLU	% CV	Note
#1	1 1	305		Liver
	1 2	928		
	[redacted]	[redacted]	7[redacted]	
	2 1	716		Lung
	2 2	242		Heart
	[redacted]	[redacted]	[redacted]	
	3 1	6930		TAIL
	3 2	334		Liver
	[redacted]	[redacted]	[redacted]	
#2	4 1	293		
	4 2	77		Lung
	[redacted]	[redacted]	82[redacted]	
	5 1	111		Heart
	5 2	247800		TAIL
	~~MEAN~~	~~123956~~	~~141.3~~	
	6 1	76992		Liver
	6 2	67295		
#3	[redacted]	[redacted]	[redacted]	
	7 1	2359		~~Heart~~
	7 2	533		Heart
	[redacted]	[redacted]	[redacted]	
	8 1	1502		TAIL
	8 2	1757		Liver
	[redacted]	[redacted]	[redacted]	
#4	9 1	1638		Liver
	9 2	160		Lung
	[redacted]	[redacted]	[redacted]	
	10 1	81		Heart
	11 1	150089		Liver
	11 2	121550		Liver
#5	[redacted]	[redacted]	[redacted]	
	12 1	1761		Lung
	12 2	552		Heart
	[redacted]	[redacted]	[redacted]	
	13 1	2156		TAIL
	13 2	131955		Liver
	[redacted]	[redacted]	[redacted]	
#6	14 1	88806		Liver
	14 2	1857		Lung
	[redacted]	[redacted]	[redacted]	
	15 1	762		heart
	15 2	2320		tail
	[redacted]	[redacted]	[redacted]	
	16 1	336		Liver
	16 2	476		Liver
	[redacted]	[redacted]	[redacted]	
#7	17 1	77		Lung
	17 2	175		heart
	[redacted]	[redacted]	[redacted]	
	18 1	243		tail
	18 2	386		Liver
#8	[redacted]	[redacted]	[redacted]	
	19 1	254		Liver
	19 2	162		Lung
	[redacted]	[redacted]	[redacted]	
	20 1	63		heart
	20 2	284		tail
	[redacted]	[redacted]	[redacted]	
#9	21 1	1324		Liver
	21 2	1523		Liver
	[redacted]	[redacted]	[redacted]	
	22 1	101		Lung
	22 2	239		heart
	[redacted]	[redacted]	[redacted]	
	23 1	199		tail
	23 2	44023384		Standards
	MEAN	22011792	141.4	
	24 1	41309624		
	24 2	215		
	MEAN	20654920	141.4	

BKG

Naked DNA

Polyplex

MC00016

MC00016

REPEAT MOUSE INJECTIONS ON MC 16 COMPACTED DNA

COMPLEX 200 μgs CMV-LUC WITH 378 μgs (3x+/-) POLY-L-LYSINE.

COMPLEX 200 μgs CMV-LUC WITH 400 μgs (3x+/-) MC00016

COMPLEX 200 μgs CMV-LUC WITH 1.2 mgs HISTONE H1 (5x +/-)

200 μgs 'NAKED' CMV-LUC AS CONTROL

INJECT 50 μgs OF EACH IN 2.5 mls RINGERS SOLUTION

mouse 1 - 50 μgs DNA

mouse 2 - DEATH

mouse 3 - ~~MC16~~ PLL 34 + 50 μg DNA

mouse 4 - ~~MC16~~ PLL 34 + 50 μgs DNA

mouse 5-6 = MC 16 + DNA

mouse ~~7~~-8 = H1 + DNA

LOW PRESSURE

mouse 9-10 = NAKED DNA IN 500λ RINGERS

mouse 11-12 = MC16 + DNA in 500λ RINGERS

12 2	596	Liver	
MEAN	42463		139.4
13 1	977	Liver	
13 2	173	Lung	
MEAN	575		98.9
14 1	120	Heart	
14 2	4050	Tail	
MEAN	2085		133.3

MC-00016

#12

15 1	543	Liver	
15 2	172	Liver	
MEAN	358		73.4
16 1	96	Lung	
16 2	33036	Heart	
MEAN	16566		140.6
17 1	35122	tail	
17 2	5642362	Rev Liver	
MEAN	2838742		139.7

LOW PRESSURE

[signature]

JAN 21, 1999

Project: IM IV (InVasc) Gut Other: EXP#: INVASC HP #11 Date: 1-21-99

Method: (LPTail) (HPTail) LPPortal HPPortal LPGut HPGut IM — 2.5 mls

Charge to: ATP PrdDev Other

Researcher(s): S.M.

Particle/Compound Being Tested:
50 mg pDNA vs H1 vs
Polyglycanic vs MC16
MC16 vs

Delivery Medium: Saline PBS Glu Man H2O (Ringers) other

Stability Issues?

Hazardous Material?

Other Cautions?

Time Point(s): 24 Hours

# of Samples: 6 x 2 = 12 (6 Samples, 12 Animals)

Explanation/Code:

End Result: Expression: (Luciferase) BetaGAL GFP Ligand/SolRecep Ab Screen other

Distribution: Fluorescence Radiation other

Organs to Assay: LUNG TAIL
LIVER LN/MC HEART

Notes:

Procedure Notes:

Start Time:

Individual Animals:

ANIMALS
pDNA < 1 - 2½ ml fast
2 - 1½ ml died during second attempt

PLL < 3/4 - 2½ Great inj
- 2½ Great inj

MC16 < 5 - OK
6 - Great

H1 < 7 2 ml then 15 min later ½ ml more
8 2 tries then in

pDNA < 9 LV 500 ml great inj
10 LV 500 ml great inj

MC16 < 11 LV 500 ml great inj
12 LV 500 ml great inj

Procedure Performed by:

Date:

LB 9507 SER. # 1088

MEASUREMENT ROUTINE

22 JAN 99 17:54 V.2.03

PROTOCOL NO. : 5	NAME :	50/50-10S
VOLUME INJ. 1 [ul]		50
VOLUME INJ. 2 [ul]		50
SEQUENCE OF INJECTIONS		1->2
DELAY TIME INJ 1/INJ 2 [s]		2.0
MEASURE BACKGROUND		YES
MEASURING TIME BKG [s]		0.5
AUTOMATIC BKG SUBTRACT		NO
MAX. BACKGROUND [RLU/s]		50
DELAY LAST INJ./MEAS. [s]		0.5
MEASURING TIME [s]		10.0

COMMENT : TAIL

SAMPLE	RLU		% CV
#1 1 1	1108703	Liver	
1 2	866981	Liver	
MEAN	~~987842~~		~~12.3~~
2 1	14332	Lung	
2 2	3156	Heart	
MEAN	~~8744~~		~~98.4~~
3 1	2589	TAIL	
3 2	121867	Liver	
MEAN	~~62228~~		~~185.5~~
#3 4 1	67106	Liver	
4 2	1176	Lung	
MEAN	~~34141~~		~~196.6~~
5 1	963	Heart	
5 2	212	tail	
MEAN	~~588~~		~~98.4~~
6 1	35925	Liver	
#4 6 2	31202	Liver	
MEAN	~~33564~~		~~10.0~~
7 1	1549	Lung	
7 2	227	Heart	
MEAN	~~888~~		~~185.8~~
8 1	1289522	Liver	
8 2	882382	Liver	
#5 MEAN	1085952		26.5
9 1	14264	Lung	
9 2	3845	Heart	
MEAN	9055		81.4
10 1	29586	TAIL	

Naked DNA 50 ug 2.2 ml Hi Pressue

Poly Lysine

MC00016

Mouse tail injections

#6 Liver 11 2	7184168	Liver		
MEAN	~~3606877~~			~~140.3~~
11 1	6394604	Liver		
1 1	86387	Lung		
1 2	20830	Heart		
MEAN	53609			86.5
2 1	14675	Tail		
2 2	482	Liver		
MEAN	~~7529~~			~~132.4~~
#7 3 1	2743	Liver		
3 2	242	Lung		
MEAN	~~1493~~			~~118.5~~
4 1	156	Heart		
4 2	1035	tail		
MEAN	~~596~~			~~104.4~~
5 1	380	Liver		
#8 5 2	315	Liver		
MEAN	~~348~~			~~13.2~~
6 1	124	Lung		
6 2	144	Heart		
MEAN	134			10.6
7 1	4339	tail		
7 2	962	Liver		
MEAN	~~2651~~			~~98.1~~
#9 8 1	446	Liver		
8 2	147	Lung		
MEAN	297			~~78.8~~
9 1	470	Heart		
9 2	404270	tail		
MEAN	~~202370~~			~~111.1~~
10 1	219	Liver		
#10 10 2	342	Liver		
MEAN	281			31.0
11 1	103	Lung		
11 2	98	Heart		
MEAN	101			3.5
12 1	84330	tail		

MC00016

Histone H1

Low Pressure 50 ug DNA

MC16 - BARN 4 HIGH PRESSURE INJECTIONS AND CONTROLS

MC 55 + MC 56 ARE NOT SOLUBLE IN HEPES BUFFER - SO ALL FORMULATION WILL BE ORIGINALLY DONE IN DMSO.

DUPLICATE MICE - 100μg DNA - pCI LUX EACH MOUSE - COMPACT 200μl EACH AGENT

1+2 = 200μg DNA + 300μl DMSO → ADD 2.5 mls RINGERS

MC16=1.7x 3+4 = 200μg DNA + 300μl DMSO + MC16-4 [3x CHARGE] 1mg → ADD 2.5mls RINGERS

5+6 = 200μg DNA + 300μl DMSO + MC16-5 [3x +] 1mg → ADD 2.5mls RINGERS

7+8 = 200μg DNA + 300μl DMSO + MC55 [~3x +] 1mg → ADD 2.5 mls RINGERS

9+10 = 200μg DNA + 300μl DMSO + MC56 [~3x +] 1mg → ADD 2.5 mls RINGERS

MC57 = 0.56x 11+12 = 200μg DNA + 300μl DMSO + MC57 [~3x +] 336μg → ADD 2.5 mls RINGERS

MC58 = 0.54x 13+14 = 200μg DNA + 300μl DMSO + MC58 [~3x +] 324μg → ADD 2.5mls RINGERS

MC59 = 0.79 15+16 = 200μg DNA + 300μl DMSO + MC59 [~3x +] 474μg → ADD 2.5mls RINGERS

MC60 = 0.78 17+18 = 200μg DNA + 300μl DMSO + MC60 [~3x +] 468μg → ADD 2.5mls RINGERS

→ FORMULATION PROBLEMS
MC16-5 IS NOT SOLUBLE - IT IS A MASS OF LARGE AGGREGATES.
MC55 FALLS OUT OF SOLUTION WHEN IT HITS RINGERS
MC59 CLOUDS UP WHEN IT HITS RINGERS

INJECTIONS

RESULTS - OVER →

1- GREAT
2- LOST SOME FORMULATION
3- GREAT
4- A LITTLE SLOW ~ 10-12 SEC STOPPED BREATHING
5- FORMULATION KILLED IT - GOOD INJECTION
6- GREAT INJECTION
7- 2.2 ml ONLY
8- GREAT INJ.
9- GOOD INJ
10- GOOD INJ - SOME PROBLEMS

11- GREAT
12- GREAT

13- GREAT INJ
14- GREAT INJ
15- GREAT INJ
16- GREAT INJ
17- GREAT INJ
18- GREAT INJ

[signature] FEB 22, 1999

Project: IM IV (InVasc) Gut Other: EXP#: InvascHP #12 Date: 2/22/99

Method: LPTail (HPTail) LPPortal HPPortal LPGut HPGut IM | Charge to: ATP PrdDev Other

Researcher(s): S.M. / LOOMIS

Particle/Compound Being Tested:
MIRUS COMPOUNDS
M16-4 M55 M57 M59 Vs pDNA
M16-5 M56 M58 M60

Delivery Medium: Saline PBS Glu Man H2O Ringers (other) DMSO

Stability Issues? —

Hazardous Material? —

Other Cautions? —

Time Point(s): 24 HOURS - HARVEST

# of Samples: 9 x 2 = 18 ANIMALS

Explanation/Code:

End Result: Expression: (Luciferase) BetaGAL GFP Ligand/SolRecep Ab Screen other

Distribution: Fluorescence Radiation other

Organs to Assay: LIVER LR/ML LUNGS KIDNEYS
SPLEEN HEART TAIL

Notes: HI VOLUME TAIL
★ WITH DMSO - ANIMAL SNOOZE AFTER INJ.

Procedure Notes:
5 WK OLD

Start Time:

Individual Animals:

ANIMALS - Inj' Harvest observations

100 ug pDNA < #1 Great / #2 Some out 1st hole > MAJOR DAMAGE to Liver #2

M16-4 < #3 Great / #4 Some of slow > Some DAMAGE - Spots

M16-5 < #5 DEAD / #6 Slow recovery > Clots

M55 < #7 2.2 ml - Blood Clots / #8 Great Light Color Spots

M56 < #9 Good inj - Shaking after inj, Blood Clots / #10 Good inj - Very Ugly Liver

M57 < #11 Great no convulsion / #12 Great Shaking > large clot on liver white spots

M58 < #13 Great > Small clots / #14 Great

M59 < #15 / #16 > Great injection →

#17 Great inj
#18 Great - Dead AM

(Also Refr. to Book PAGE 141)

Procedure Performed by: Mark Noble

Date: 2/22/99

2/24/99 Samples brought 23rd frozen received 24th

INVASC HP #12

2.5 ml High Volume & pressure

2/24/99

9507 SER. # 1088

MEASUREMENT ROUTINE

24 FEB 99 09:09 V.2.03

PROTOCOL NO. : 8 NAME :	10
VOLUME INJ. 1 [ul]	50
VOLUME INJ. 2 [ul]	50
SEQUENCE OF INJECTIONS	1->2
DELAY TIME INJ 1/INJ 2 [s]	2.0
MEASURE BACKGROUND	YES
MEASURING TIME BKG [s]	0.5
AUTOMATIC BKG SUBTRACT	NO
MAX. BACKGROUND [RLU/s]	50
DELAY LAST INJ./MEAS. [s]	0.5
MEASURING TIME [s]	10.0

COMMENT : DR.M

SAMPLE	RLU	
1	26050686	LR > Liver
2	21630646	mc (Animal)
3	5847298	Spleen (100 ng Naked DNA)
4	1014409	Lung
5	560690	Heart
6	694563	kidneys
7	13254	tail
8	1566483	LR > liver (animal #2)
9	1191401	mc (Liver damaged trashed!)
10	516558	Spleen
11	85148	Lung (100 ng Naked DNA)
12	68804	Heart
13	28135	kidneys
14	36783	tail

S.M. Complexes

24 Hour harvest

LIVERS 10x dilution each!

15	12076003	LR > LIVER	
16	8672595	mc	
17	3967398	Splem	#3
18	649693	Lung	MC16-4
19	358458	Heart	
20	88135	Kidneys	
21	22478	tail	
22	20836924	LR	
23	14335173	mc	#4
24	10303277	Spleen	MC16-4
25	2205514	Lung	
26	319212	Heart	
27	435213	kidneys	
28	34322	tail	
29	15893273	LR	#6 - [redacted]
30	14260842	mc > LIVER	MC16-5 d during
31	3568655	Spleen	ht
32	663279	Lung	
33	212093	Heart	
34	2751592	kidneys	pleura
35	7972	tail	

#5 dead - [redacted] ml

3 pDNA

INVASC NP #12

	MC-55 ↓		
	36	17862760	LR > Liver
#7	37	15428035	MC
	38	356868	Spleen
MC55	39	392596	Lung
	40	215870	Heart
	41	75095	Kidney
	42	2859	Tail
	43	13063372	LR
#8	44	13417665	MC > Liver
MC55	45	4958813	Spleen
	46	785182	Lung
	47	231348	Heart
	48	184282	Kidney
	49	63918	Tail

	MC-56 ↓		
	50	5976220	LR > Liver
#9	51	5326380	MC
MC-56	52	1342129	Spleen
	53	1463423	Lung
	54	128175	Heart
	55	106433	Kidney
	56	26504	Tail
	57	2482764	LR
#10	58	1433567	MC > Liver
MC-56	59	499820	Spleen
	60	113231	Lung
	61	63804	Heart
	62	88860	Kidney
	63	191769	Tail

	MC-57 ↓		
	64	6892050	LR > Liver
#11	65	5883566	MC
	66	2794699	Spleen
	67	261024	Lung
	68	122821	Heart
	69	151890	Kidney
	70	30516	Tail
#12	71	17868750	LR > Liver
	72	11401863	MC
	73	4176665	Spleen
	74	678634	Lung
	75	375063	Heart
	76	186018	Kidney
	77	5061	Tail

	MC-58 ↓		
#13	78	30968612	LR > Liver
	79	23492376	MC
	80	7578113	Spleen
	81	1440903	Lung
	82	802760	Heart
	83	624041	Kidney
	84	12032	Tail
#14	85	11969210	LR > Liver
	86	7973374	MC
	87	1864613	Spleen
	88	300536	Lung
	89	251567	Heart
	90	363527	Kidney
	91	11889	Tail

	MC-59 ↓		
#15	92	78586	LR > Liver
	93	54733	MC
	94	114178	Spleen
	95	35211	Lung
	96	40888	Heart
	97	48360	Kidney
	98	9235	Tail
#16	1	285662	LR > Liver
	2	262457	MC
	3	16426	Spleen
	4	10562	Lung
	5	15567	Heart
	6	9187	Kidney
	7	4811	Tail

	MC-60	#17	
#17	8	769642	LR > Liver
	9	669180	MC
	10	13547	Spleen
	11	15585	Lung
	12	2570	Heart
	13	12769	Kidney
	14	325391	Tail

Number 18 died during night

2/24/99
S.M. Complexes
High Volume
2.5 ml
100 ng pDNA